JOURNAL OF VIROLOGY, Apr. 2005, p. 4043–4054 Vol. 79, No. 7
0022-538X/05/$08.00+0 doi:10.1128/JVI.79.7.4043–4054.2005

Divergent Host Responses during Primary Simian Immunodeficiency Virus SIVsm Infection of Natural Sooty Mangabey and Nonnatural Rhesus Macaque Hosts

Guido Silvestri,[1,2] Andrew Fedanov,[2] Stephanie Germon,[2] Natalia Kozyr,[2] William J. Kaiser,[2] David A. Garber,[1,2] Harold McClure,[3]† Mark B. Feinberg,[1,2,4] and Silvija I. Staprans[1,2,4]*

Departments of Medicine[1] and of Microbiology and Immunology[4] and Emory Vaccine Center,[2] Emory University School of Medicine, and Yerkes National Primate Research Center of Emory University,[3] Atlanta, Georgia

Received 5 October 2004/Accepted 11 November 2004

To understand how natural sooty mangabey hosts avoid AIDS despite high levels of simian immunodeficiency virus (SIV) SIVsm replication, we inoculated mangabeys and nonnatural rhesus macaque hosts with an identical inoculum of uncloned SIVsm. The unpassaged virus established infection with high-level viral replication in both macaques and mangabeys. A species-specific, divergent immune response to SIV was evident from the first days of infection and maintained in the chronic phase, with macaques showing immediate and persistent T-cell proliferation, whereas mangabeys displayed little T-cell proliferation, suggesting subdued cellular immune responses to SIV. Importantly, only macaques developed CD4$^+$-T-cell depletion and AIDS, thus indicating that in mangabeys limited immune activation is a key mechanism to avoid immunodeficiency despite high levels of SIVsm replication. These studies demonstrate that it is the host response to infection, rather than properties inherent to the virus itself, that causes immunodeficiency in SIV-infected nonhuman primates.

Infections with CD4$^+$-lymphocyte-tropic lentiviruses are prevalent in numerous nonhuman primate species in Africa. Despite lifelong simian immunodeficiency virus (SIV) infection and high-level SIV replication, natural reservoir hosts maintain normal CD4$^+$-T-cell counts and avoid AIDS (2, 49, 53). In contrast, human immunodeficiency virus (HIV) infection in humans and experimental SIV infection of nonnatural Asian macaque hosts (such as rhesus macaques [RMs]) leads to progressive CD4$^+$-T-cell depletion and AIDS. Understanding the basis of the nonpathogenic host-virus relationship in natural hosts is likely to provide important clues regarding AIDS pathogenesis.

In pathogenic HIV and SIV infections, "set-point" levels of virus replication are directly correlated with rates of CD4$^+$-T-cell decline and disease progression (36, 57, 60). Studies from HIV-infected humans and SIV-infected macaques indicate that CD8 T-cell-mediated responses may, at least transiently, constitute potent antiviral responses against infection with CD4 T-cell-tropic lentiviruses and thus appear to be beneficial to the host (8, 59). However, these responses are ultimately ineffective in controlling viral replication in the vast majority of infected individuals, resulting in chronic high antigenemia.

It has long been recognized that pathogenic lentivirus infections are associated with the progressive loss of uninfected CD4$^+$ and CD8$^+$ T cells; this is mainly due to excessive activation-induced cell death (10, 19, 37). This elevated apoptosis was thought to arise from the chronic high levels of immune activation that follow HIV infection (19, 42). The observation that levels of CD8 T-cell activation predicted the rates of disease progression as well as, or even better than, viral load itself did (15, 25, 54), supported the notion that infection-elicited T-cell activation on its own had a detrimental effect on the host immune system (16, 21).

More recent studies have helped to elucidate that HIV infection results in the accelerated activation and proliferation of the effector-memory subset of T cells (22). Expansion of these cells likely leads to the observed activation-induced cell death of large numbers of uninfected "bystander" CD4 and CD8 T lymphocytes and to the direct HIV infection of CD4 T cells (20, 52). This HIV-induced immune activation likely also interferes with the proper generative function of bone marrow, thymic, and peripheral sites of lymphocyte proliferation and differentiation and thus to an inability to replace lost CD4$^+$ T cells (34, 50). Expansion of effector-memory cells is thought to exert a drain on the naive T-cell pool and to prevent effective establishment of a normal pool of central memory cells (52), leaving the host unable to generate either new primary responses or effective recall responses, including responses to HIV infection (38). The continuous production of proinflammatory and proapoptotic cytokines by the expanded effectors likely amplifies the bystander immunopathology associated with HIV infection (13). Combined, these studies of immunodeficiency virus infection of nonnatural hosts suggest that chronic immune activation and increased turnover of certain T-cell subsets play a prominent role in the pathogenesis of AIDS.

Naturally occurring examples of persistently viremic yet disease-free infections remain largely unstudied. In order to understand the host-virus equilibrium that avoids disease, we have been analyzing naturally SIVsm-infected sooty mangabeys (SMs), the source of the human HIV type 2 (HIV-2)

* Corresponding author. Mailing address: Emory Vaccine Center, 954 Gatewood Rd., NE, Atlanta, GA 30329. Phone: (404) 727-8214. Fax: (404) 727-8199. E-mail: sstapr2@sph.emory.edu.
† Deceased.

epidemic (7). The high-level viremia in infected SMs (53) indicates that SIVsm is not effectively controlled by the humoral or cellular immune responses elicited during infection, an observation consistent with the limited frequency of SIV-specific cytotoxic-T-lymphocyte (CTL) responses in vivo (29). To address whether SIV infection is cytopathic for SM CD4 T cells in vivo, we have measured the turnover of virus and infected cells by using the same methods that have been used in HIV-infected patients and SIV-infected RMs (24, 44, 61). These studies indicate that SIVsm viremia is maintained by multiple rounds of de novo infection of CD4 T cells, whose longevity is estimated to be as short as the infected CD4 T cells present in pathogenic HIV and SIV infections (R. M. Grant and M. B. Feinberg, unpublished data); thus, the absence of disease is not explained by a lack of SIVsm cytopathicity for SM $CD4^+$ T cells. Rather, SIVsm infection of SMs is characterized by low levels of aberrant immune activation and apoptosis compared to pathogenic HIV and SIV infections (53). In particular, SIVsm-infected SMs manifest little if any increased CD8 T-cell turnover, as is characteristic of HIV-infected humans (53). Thus, attenuated T-cell activation appears to enable SMs to avoid the bystander damage seen in pathogenic infections and to maintain preserved T-lymphocyte populations and regenerative capacity. An important question is how chronically SIVsm-infected SMs come to manifest this subdued cellular response to SIV infection. In a well-characterized murine model of an anergic cellular immune response to lymphocytic choriomeningitis virus infection, an initial massive CD8 T-cell response to the virus is followed by exhaustion of the responding CD8 T-cell clones (45).

We reasoned that the comparative study of the initial interactions of SIV with natural and nonnatural hosts could elucidate key cellular differences underlying divergent host responses and infection outcome. To directly compare natural and nonnatural host responses to SIV, it was necessary to identify natural and nonnatural hosts that could be readily infected with the same virus. Although SIVs have been isolated from numerous natural simian hosts, most of these viruses have not been characterized with respect to their ability to readily infect new nonnatural hosts. An SIVagm strain derived from a naturally infected African green monkey (AGM) induced an AIDS-like syndrome in pig-tailed macaques but, upon reintroduction into AGMs, the virus established a low-level viremia that is not characteristic of naturally infected AGMs (23). Similarly, the molecularly cloned SIVmac239 derived from extensive passage of SIVsm in RMs and that is highly pathogenic in them replicated poorly when reintroduced into SMs (26). In an attempt to establish a comparative SIV infection model in which both natural and nonnatural hosts could be robustly infected with an identical SIV inoculum, we inoculated SMs and RMs with unpassaged SIVsm obtained from a naturally infected SM. SIVsm infection resulted in substantial virus replication in both SMs and RMs and recapitulated the chronic high viremia seen in naturally infected SMs. However, only the nonnatural RM host developed chronic T-cell activation, CD4 T-cell loss, and AIDS. This model demonstrates that the primary determinant of whether or not disease follows infection is the nature of the host response to SIV infection and not the virus itself.

MATERIALS AND METHODS

Animals and infections. SMs (*Cercocebus atys*) and RMs (*Macaca mulatta*) were housed at the Yerkes National Primate Research Center and maintained in accordance with federal guidelines (43). Prior to study entry, the absence of SIV infection was confirmed by negative SIV PCR of plasma and negative HIV-2 serology for at least 1 year. Three uninfected SMs (aged 4 to 8 years, one male and two females) and three uninfected RMs (ages 5 to 6 years, all males) were infected intravenously (i.v.) with 1 ml of plasma obtained from a naturally SIVsm-infected SM (animal FQi, age 12 years, infected with SIV since age 4 years).

SIVsm RNA quantitation. Viral RNA was directly extracted from 150 μl of acid-citrate-dextrose anticoagulated plasma by using QIA Amp Viral RNA kits (Qiagen, Valencia, Calif.), eluted in 55 μl of water, and frozen at −80°C until batch SIV RNA quantitation was performed. Random hexamers, rather than sequence-specific primers, were used to prime reverse transcription of the diverse quasispecies of SIVsm viral RNA present in naturally infected SMs. Next, 5-μl aliquots of purified plasma RNA were reverse transcribed in a final 20-μl volume containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 5 mM $MgCl_2$, 1 μM concentrations of each deoxynucleoside triphosphate, a 0.5 μM concentration of forward primer, a 0.5 μM concentration of reverse primer, a 0.1 μM concentration of probe, and 5 U of AmpliTaq Gold DNA polymerase (all reagents from Applied Biosystems, Foster City, Calif.). The primer sequences within a conserved portion of the SIV *gag* gene are the same as those described previously (55). An ABI PRISM 7700 sequence detection system (Applied Biosystems) was used with the following PCR cycling profile: 95°C for 10 min, followed by 40 cycles at 93°C for 30 s and 59.5°C for 1 min. PCR product accumulation was monitored by using a probe to an internal conserved gag gene sequence: 5′-FAM (6-carboxyfluorescein)-CTGTCTGCGTCATTTGGTGC-TAMRA (6-carboxyltetramethylrhodamine), where FAM and TAMRA denote the reporter and quencher dyes, respectively. The SIV RNA copy number is determined by comparison to an external standard curve consisting of virion-derived SIVmac239 RNA previously quantified by the SIV bDNA method (P. Dailey, unpublished data). All specimens are extracted and amplified in duplicate, with the mean result reported (53).

Flow cytometry for cell surface markers. Hematological studies and cell separation were performed as described previously (53). Peripheral blood mononuclear cells were analyzed by double- or triple-color fluorescent antibody staining to determine the percentage and absolute number of specific cell subpopulations as previously described (53).

Humoral responses. Serum samples were assessed for antibodies to SIV proteins by Western blotting as previously described (4).

Histology and immunohistochemistry. Sections were cut from paraffin-embedded formalin- or paraformaldehyde-fixed tissues, deparaffinized, and stained with hematoxylin and eosin. Proliferating cells were detected with Ki67 antibody (Dako, catalog no. A0047) as described previously (12). Apoptotic cells were identified by detection of DNA fragmentation in situ according to the manufacturer's instructions (Intergen Company, catalog no. S7110).

ISH. SIV in situ hybridization (ISH) of formaldehyde or paraformaldehyde-fixed, paraffin-embedded tissues was performed as described previously (62) by using a riboprobe complementary to the 5′ 1.1-kb portion of the cloned SIVmac239 provirus.

Statistical methods. Statistical analyses of viral load were performed on $\log_{10}$-transformed SIV RNA values. Calculation of the rates of viral growth and decay during primary infection was performed as described previously (57). Correlations involving different sets of data (i.e., the various immunophenotypic and viremia variables under study) were determined by using the standard Pearson or Spearman correlation coefficients.

RESULTS

Uncloned SIVsm from a naturally infected SM replicates to high levels in SMs and RMs. Although existing SIV strains commonly used in macaque models were derived by direct transfer (experimental or accidental) of SIVsm from SMs into macaques, the initial events surrounding experimental cross-species inoculation of SIV, such as the efficiency of establishing infection, the viral replication dynamics, or virus evolution in the new host, have not been characterized. In this experiment, three SMs and three RMs were i.v. inoculated with 1 ml of SM plasma (containing 4×10^6 SIVsm RNA copies/ml) obtained

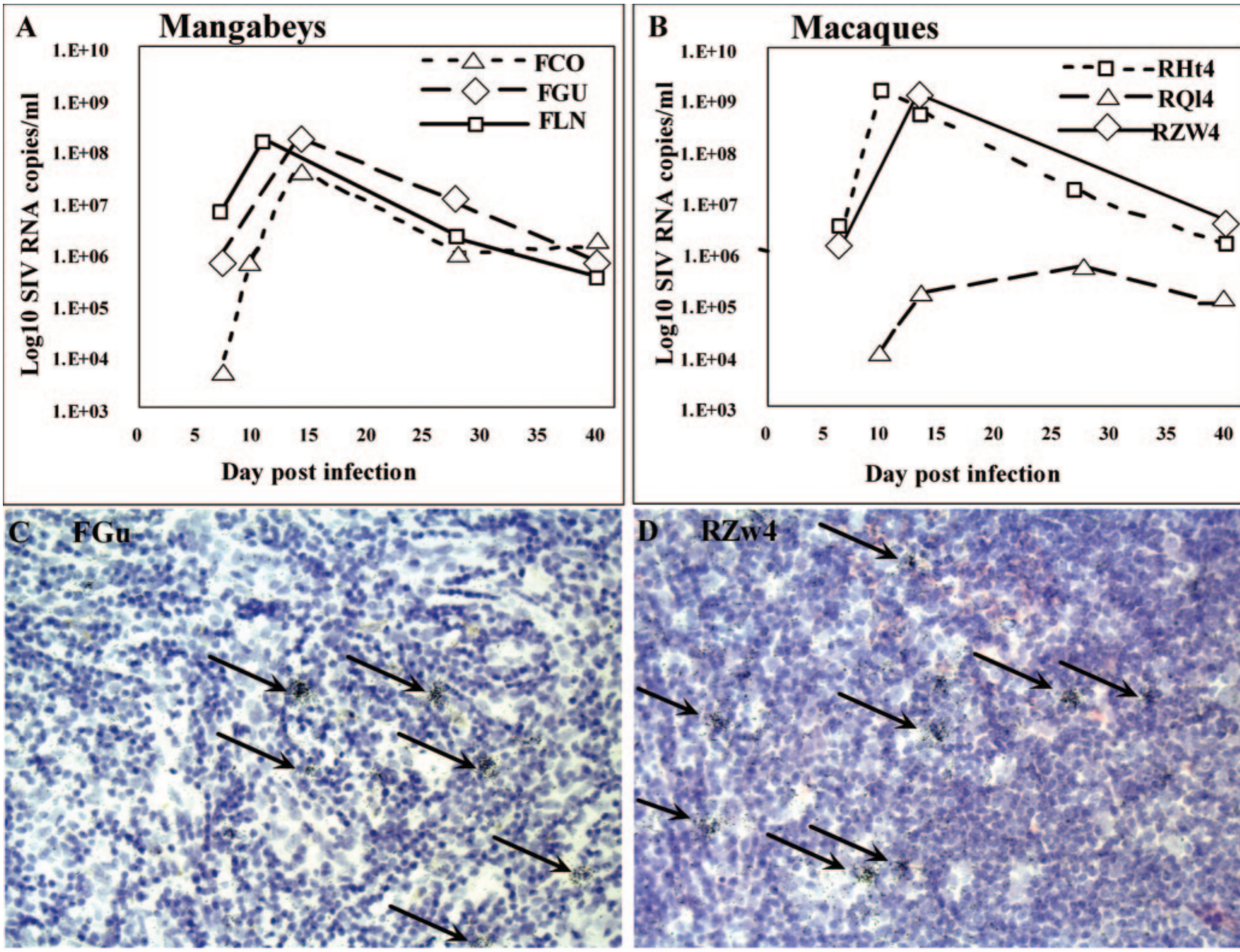


FIG. 1. Viral replication during primary SIVsm infection of RMs and SMs. (A and B) SIV plasma viremia in the three SMs (A) and the three RMs (B). (C and D) SIV replication was detected by ISH in a lymph node biopsy from a SM (FGu) and a RM (RZw4) at day 14 postinfection.

from a healthy naturally infected SM. i.v. inoculation was used to reliably infect and to compare SIVsm replication dynamics and the divergent host responses to an identical virus inoculum in the two species. i.v. inoculation likely differs from the circumstances of natural SIVsm transmission among SMs, which is thought to occur through sexual routes, whereas zoonotic or cross-species transmission of SIV to new hosts is hypothesized to involve exposure to blood or bloody flesh (11, 46).

By using a quantitative reverse transcription-PCR method that accurately enumerates the highly diverse SIVsm quasispecies in naturally infected animals (53), we observed robust SIVsm replication in all three SMs (Fig. 1A). Peak virus levels in the SMs were $>10^7$ SIV RNA copies/ml, followed by setpoint levels between 10^5 and 10^6 SIV RNA copies/ml. Thus, experimental inoculation of uncloned SIVsm into SMs achieved chronic viremia levels identical to those observed in naturally infected SMs (49, 53). Interestingly, this unpassaged SIVsm displayed early and vigorous replication in the new RM hosts (Fig. 1B), with two of three animals experiencing peak viremia levels of 2×10^9 (RHt4) and 6×10^8 (RZw4) SIV RNA copies/ml. These peaks of viremia in RMs are up to 40-fold higher than those observed in the natural SM host. One RM (RQl4) experienced a more delayed and less robust pattern of virus replication; peak levels of viremia likely occurred between 14 and 28 days postinfection (an interval when no sampling was performed) and likely exceeded the 5×10^5 SIV RNA copies/ml observed at day 28.

Previous studies of pathogenic primary HIV and SIV infection of humans and RMs have demonstrated a rapid initial viral doubling time of about 7 h in newly infected hosts. Appropriately timed (biweekly) samples were available during the early viral rise for one RM (RHt4) and one SM (FCo) with which to estimate virus doubling times. In the SM FCo, the observed virus doubling time was 9.1 h, which is within the ranges reported for both pathogenic SIV and HIV infections (33, 44, 57). In RM RHt4, the calculated viral doubling time was 8.6 h, which is closer to the mean values reported in two different studies of SIV infection of RMs (44, 57). Thus, the initial growth rate of SIVsm upon transfer to a new host species (RMs) appears to be at least as fast as that observed in its natural SM host. Additional studies involving more frequent sampling will be required to fully document the parameters of early SIV replication that can lead to higher peak viremia in the nonnatural RM host.

SIV ISH of lymph nodes from one SM and one RM at day 14 postinfection demonstrated readily detectable SIV RNA-positive cells in both animals (Fig. 1C and D). Silver grain counts indicated that individual SIV-infected cells in RM RZw4 contained ca. 30% more grains than were observed in the lymph node from SM FGu (median grain number 180 $\pm$ 21 versus 140 $\pm$ 15, $P < 0.05$ [data not shown]). This suggests that on a per-cell basis, SIV reproduces as well, if not even better, in the new RM host as it does in the natural SM host. Taken together, these data suggest that extensive viral passaging or virus selection are not required for SIVsm to readily infect and replicate to high levels in the new RM host.

RMs and SMs respond to SIVsm infection with divergent patterns of T-cell activation and proliferation. To monitor the early response of SM and RM hosts after inoculation with SIVsm, changes in peripheral blood lymphocyte subsets were monitored by flow cytometry up to twice weekly for the first 2 weeks postinfection and then monthly thereafter. Within the first few days of SIV infection, activation of both $CD4^+$ and $CD8^+$ T cells was observed in both species (Fig. 2). $CD4^+$-T-cell activation, as assessed by the percentage and absolute number of DR^+ $CD4^+$ T cells, initially peaked at days 3 to 7 postinfection. Thereafter, peak levels of virus replication were observed at days 10 to 14 in all animals (Fig. 2A and B; representative animals are shown). Finally, a peak of $CD8^+$-T-cell activation (shown as $CD8^+$ $CD69^+$ T cells in Fig. 2A and B) occurred at days 10 to 14. Activated $CD8^+$ T cells were more abundant than activated $CD4^+$ T cells in both species, a finding consistent with previous reports in SIV-infected RMs (27).

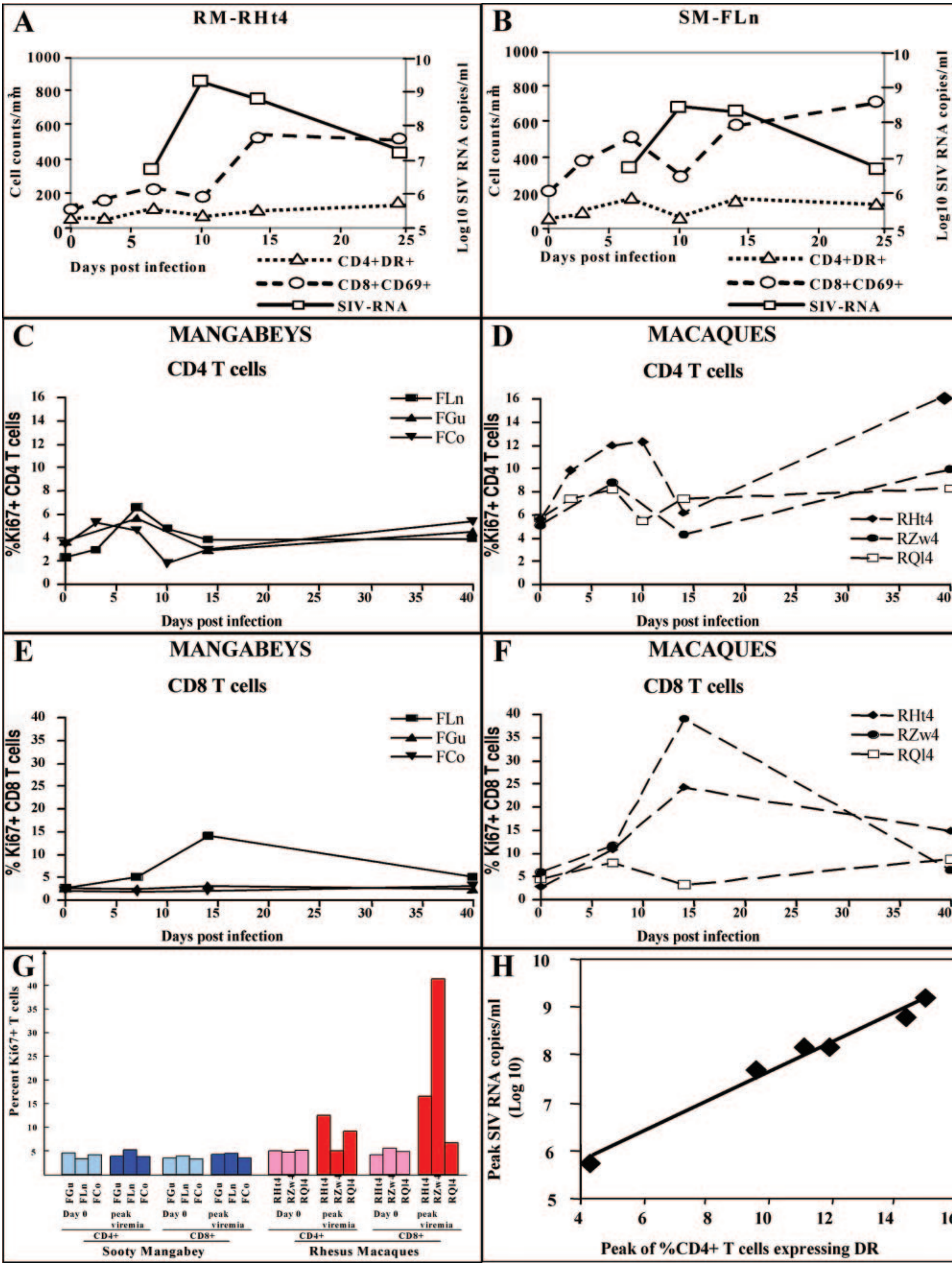
T-cell proliferation was monitored by using Ki67 as a marker of cycling lymphocytes (14, 40, 47) during acute viral infection. In recent studies of the anti-SIV $CD8^+$-T-cell mediated immune responses during acute SIVmac239 infection of $MaMuA01^+$ RMs, we have shown that the number of $CD8^+$ $Ki67^+$ T cells directly correlates with the number of SIV-specific $CD8^+$ T cells, as assessed by tetramer staining (12). Consistent with other observations (5), the three RMs manifested higher baseline levels of $CD4^+$ $Ki67^+$ T cells (5 to 6%) and $CD8^+$ $Ki67^+$ T cells (3 to 6%) than did the three SMs (2 to 4% $Ki67^+$ $CD4^+$ and $CD8^+$ T cells; Fig. 2C to F). At 7 to 10 days after SIVsm inoculation, the level of proliferating $CD4^+$ T cells increased to 8 to 13% in the RMs compared to 6 to 7% in SMs (Fig. 2C and D). In the $CD8^+$-T-cell compartment, the RMs experienced peak levels of 8 to 40% proliferating cells, followed by persistently elevated CD8 T-cell proliferation (Fig. 2F). In contrast, one of three SMs manifested a transient peak of 15% proliferating $CD8^+$ T cells, whereas the other two SMs did not manifest any appreciable increase in proliferating $CD8^+$ T cells despite similarly high levels of SIV replication (Fig. 2E). Thus, there appeared to be a species-specific divergence in the extent of $CD8^+$-T-cell proliferation induced in response to SIVsm infection. In SMs, there was a disassociation between the early expression of CD69 on $CD8^+$ T cells and subsequent $CD8^+$-T-cell proliferation. If SM $CD8^+$ T cells did proliferate in response to SIV infection, they did so to a lesser extent than in RMs, as shown by less $CD8^+$-T-cell proliferation on the day of peak viremia (Fig. 2G).

In previous studies of acute SIV infection of RMs we have observed an association between the magnitude of early CD4 T-cell proliferation and subsequent peak levels of SIV replication (12, 56). In this acute SIVsm infection of RMs and SMs the percentage of $CD4^+$ DR^+ T cells observed during the initial peak of $CD4^+$-T-cell activation (days 3 to 7) was significantly correlated with the subsequent peak levels of virus replication in plasma (Fig. 2H, $R = 0.98$, $P < 0.001$). After the acute viremia period, the positive association between $CD4^+$-T-cell activation and SIV replication disappeared, with RMs showing higher peripheral blood and lymph node levels of $CD4^+$- and $CD8^+$-T-cell proliferation than SMs, despite a slightly lower set-point viremia.

Immunohistochemical analysis of $Ki67^+$ T cells in lymph nodes of one SM and one RM at day 14 also demonstrated divergent patterns of lymphocyte activation. SM FGu manifested a preponderance of activated B cells within germinal centers, the formation of multiple secondary follicles, and a relatively quiescent T-cell zone (Fig. 3A). In contrast, RM RZw4 manifested limited B-cell activation; rather, extensive lymphocyte proliferation was observed in the T-cell zone (Fig. 3B). Lymph nodes for the RMs, but not the SMs, were also noticeably enlarged at this time. Thus, the more extensive peripheral blood T-cell proliferation observed in RMs versus SMs was also reflected in the lymphoid tissues.

RMs show a larger postpeak decline in viremia, the magnitude of which correlates with the extent of early $CD8^+$-T-cell proliferation. In pathogenic HIV or SIV infection, the postpeak decline in viremia coincides with $CD8^+$-T-cell proliferation and the appearance of virus-specific $CD8^+$ T cells (1, 12, 30, 31, 48). Although all animals in the present study seroconverted to anti-SIV antibodies, measures of SIV-specific cellular immune responses were not performed. In order to compare the extent of virus suppression following peak viremia in SMs and RMs, the magnitude of viral decline, calculated as the difference between peak and set-point viremia, was determined for both species. SMs showed 2.3- to 2.7-$\log_{10}$ decrements in viremia, resulting in similar set-point levels of viremia of 3.0×10^5 to 5.7×10^5 SIV RNA copies/ml (Fig. 4A). (Set-point viremia was calculated as the average viremia measured be-

FIG. 2. T-cell activation and/or proliferation and viral replication during acute SIVsm infection of SM and RM. (A and B) Sequential activation of CD4 T cells, SIV replication, and CD8 T-cell activation in a representative RM (RHt4) and SM (FLn). For $CD4^+$ HLA^- DR^+ T cells and $CD8^+$ $CD69^+$ T cells, the numbers represent absolute cell counts per cubic millimeter. Plasma viremia is expressed as the $\log_{10}$ SIV RNA copies/milliliter of plasma. (C to F) Rates of T-cell proliferation in $CD4^+$ (C and D) and $CD8^+$ (E and F) T cells from three SMs (C and E) and three RMs (D and F) measured as percentage of Ki67 expression between day 0 and day 40 postinfection. (G) Average percentage $Ki67^+$ CD4 and CD8 T cells on the day of infection and at the time of peak viremia. Peak viremia occurred at either day 10 (animals RHt4, FLn), 14 (animals RZw4, FCo, and FGu), or day 28 (animal RQl4). For RQl4, measures of $Ki67^+$ T cells were not available at day 28; therefore, the next measurement at day 40 was taken. (H) Direct correlation between peak of HLA-DR expression on $CD4^+$ T cells and peak plasma viremia in all six animals.


A
RM-RHt4
Cell counts/mm³
Log10 SIV RNA copies/ml
Days post infection
CD4+DR+
CD8+CD69+
SIV-RNA
B
SM-FLn
C
MANGABEYS
CD4 T cells
% Ki67+ CD4 T cells
FLn
FGu
FCo
D
MACAQUES
RHt4
RZw4
RQl4
E
CD8 T cells
% Ki67+ CD8 T cells
F
G
Percent Ki67+ T cells
Day 0
peak viremia
CD4+
CD8+
Sooty Mangabey
Rhesus Macaques
H
Peak SIV RNA copies/ml (Log 10)
Peak of %CD4+ T cells expressing DR

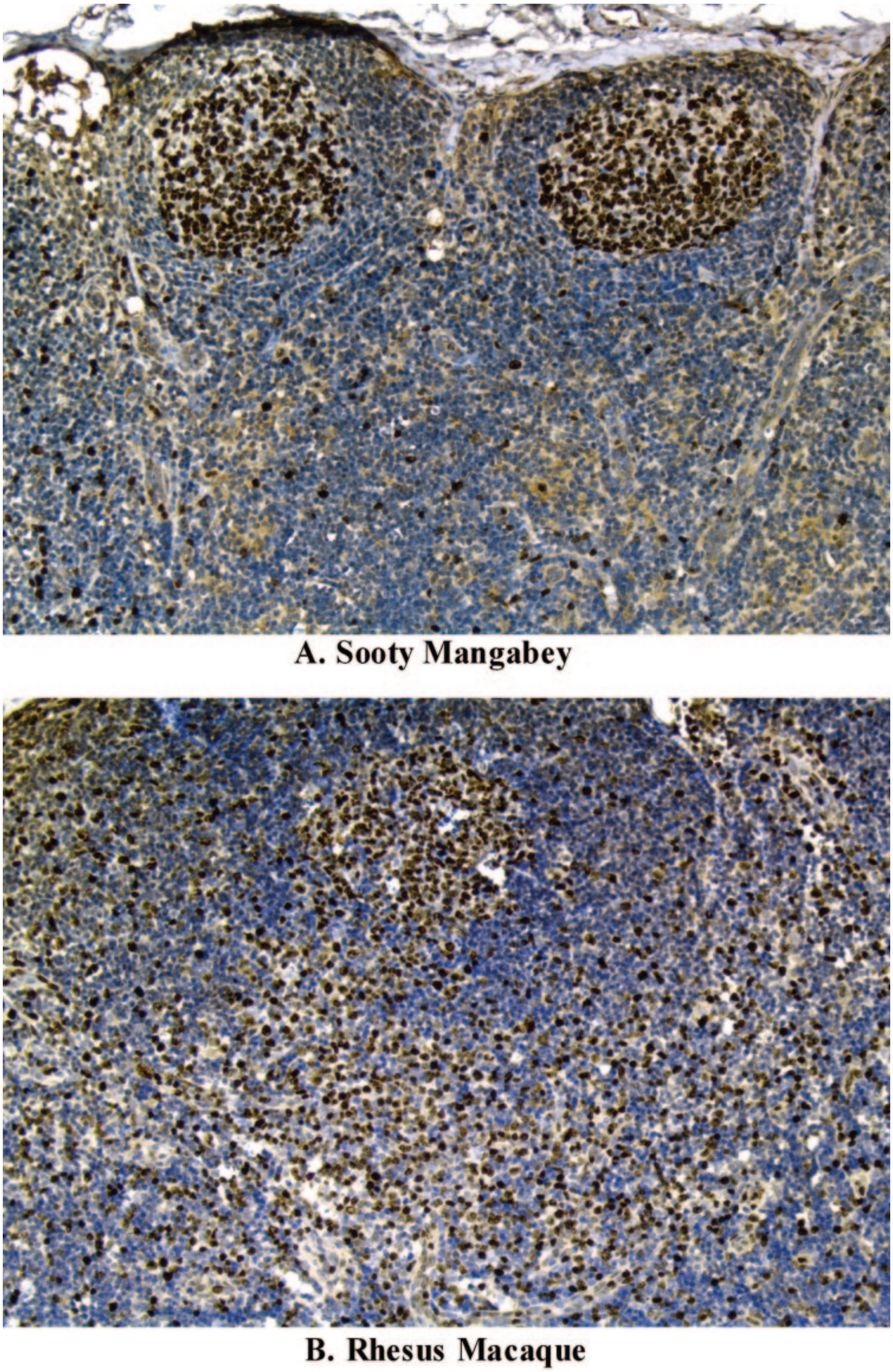


FIG. 3. Divergent lymphocyte proliferation profiles in lymph nodes during SIVsm infection of SM and RM. (A) Immunohistochemical staining with Ki67 antibody of a lymph node biopsy obtained from a representative SM (FGu) at day 14 postinfection. The vast majority of the $Ki67^+$ cells are localized in the B-cell areas, i.e., folliculi and germinal centers, whereas minimal Ki67 expression was observed in the T-cell area (i.e., paracortex). (B) Immunohistochemical staining with Ki67 antibody of a lymph node biopsy obtained from a representative RM (RZw4) at day 14 postinfection.

tween days 100 and 400 postinfection.) In contrast, RMs demonstrated 3.2- to 4.0-$\log_{10}$ decrements in viremia (Fig. 4B), resulting in somewhat lower set-point viremia levels of between 4×10^2 and 3.2×10^5 SIV RNA copies/ml. These levels are lower than those observed after infection with pathogenic RM-adapted SIVs (12, 26, 57) and more closely resemble the variation in and magnitude of viremia seen after HIV infection of humans (35, 36). Thus, SIVsm-infected RMs manifested

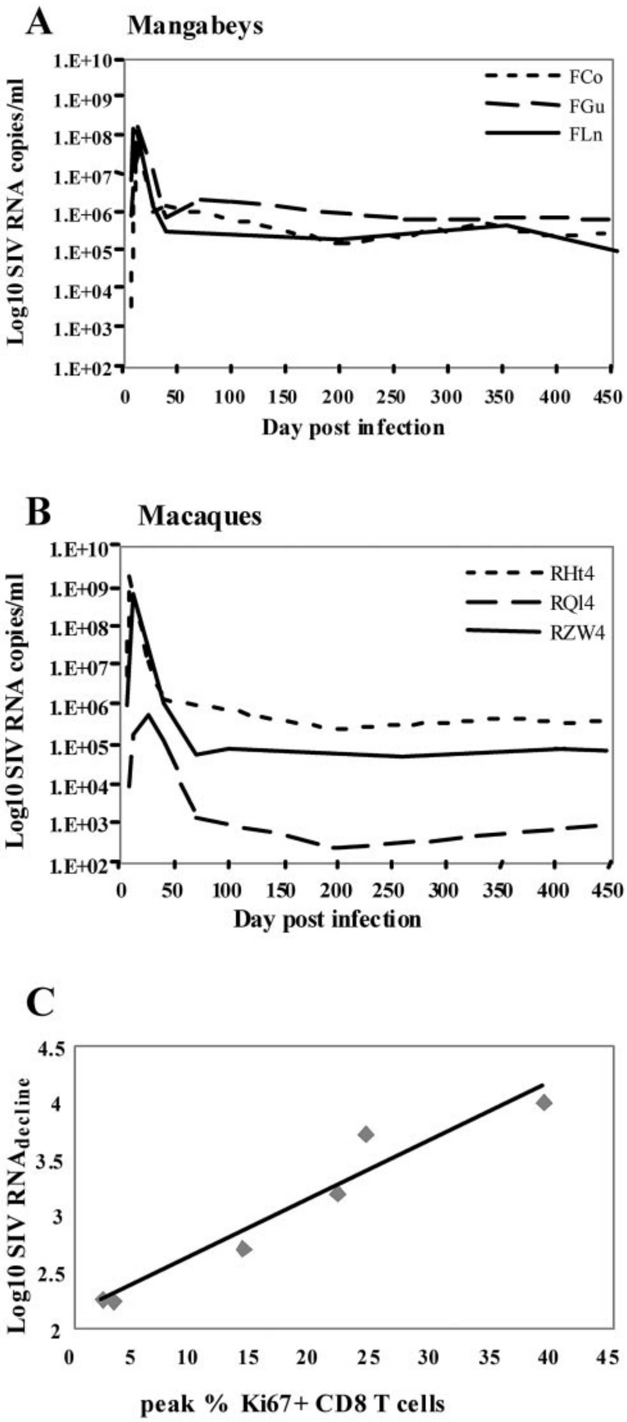


FIG. 4. Acute CD8$^+$-T-cell proliferation and establishment of postpeak levels of virus replication. (A and B) Postpeak decline of plasma viremia and establishment of levels of set-point viremia in SMs (A) and RMs (B) as measured between days 7 and 450 postinfection. (C) Correlation between rates of peak CD8$^+$-T-cell proliferation, measured as Ki67 expression, and magnitude of postpeak decline in plasma viremia in three SIVsm-infected SMs and three RMs.

both larger and more variable postpeak declines in viremia compared to infected SMs.

SIV ISH of lymphoid tissues from the peak (day 14) and one postpeak time point (day 40) for one SM and RM showed that the postpeak decline in plasma SIV RNA levels in RZw4 was accompanied by a 55-fold reduction in the frequency of SIV RNA$^+$ cells in lymphoid tissue, whereas the decline in the SIV RNA levels in plasma in FGu was accompanied by an 18-fold reduction in the frequency of SIV$^+$ cells (data not shown). Thus, in terms of both the SIV RNA levels in plasma and the frequency of infected cells in lymph nodes, the magnitude of virus suppression was greater in the RMs than in the SMs.

SIV suppression was temporally correlated with the appearance of increased CD8$^+$-T-cell proliferation in all three RMs and one SM (FLn). Correlation analyses demonstrated a significant relationship between the magnitude of peak CD8$^+$-T-cell proliferation and the magnitude of postpeak decline in viremia (Fig. 4C, $R = 0.83$, $P = 0.04$). This CD8$^+$-T-cell proliferation did not appear to be simply a response to the level of viral antigen load, since there was no relationship between peak SIV RNA levels and peak CD8$^+$-T-cell proliferation, especially in the SMs. These data are consistent with observations in a study of primary SIVmac239 infection of RMs in which the magnitude of acute CD8 T-cell proliferation reflected the strength of the antiviral immune response, as evidenced by its temporal correlation with SIV-specific CD8 T cells and the relationship between the CD8 T-cell proliferative response and virus suppression (12, 48). If the acutely proliferating CD8 T cells in the present study similarly represent an SIV-specific cellular immune response, then this would help to explain the larger postpeak suppression of viremia observed in the RMs compared to the SMs.

CD4$^+$- and CD8$^+$-T-cell proliferation and lymphocyte apoptosis remain elevated in the nonnatural RM host. To monitor longer-term trends in T-cell proliferation in the infected animals, the percentage of Ki67$^+$ T cells were evaluated at various time points after infection and compared to the values observed prior to SIV infection (Fig. 5). In SIV-infected SMs there was no evidence of chronic elevation of CD8$^+$-T-cell proliferation (Fig. 5B). A slight increase in Ki67$^+$ CD4$^+$ T cells was observed in one SM (FLn; Fig. 5A), a finding consistent with a two-fold increase in this parameter in a cross-sectional analysis of a large number of SIV-infected versus SIV-negative SMs (53). Thus, in SMs considerable levels of SIV replication (10^5 to 10^6 copies/ml) were not accompanied by increased rates of T-cell proliferation. In contrast, two of the three SIV-infected RMs showed chronic elevation of CD4$^+$- and CD8$^+$-T-cell proliferation, despite having similar or lower set-point viremia than the SMs (Fig. 5). The one RM that experienced a more attenuated course of SIV infection (RQl4) maintained baseline levels of CD4$^+$-T-cell proliferation and showed little evidence of increased CD8$^+$-T-cell proliferation; thus, this nonprogressing RM was characterized by the absence of significant T-cell proliferation during both the acute and the chronic phases of SIV infection. The low viremia and lack of immune activation in RQl4 may result from better immunological control of the infection by a more focused and effective response that does not result in a generalized state of immune activation, a lack of fitness of the inoculated virus for that specific animal, or possibly by the inherently more immunologically quiescent status of this particular animal.

Histologic analysis of lymph nodes (Fig. 6) showed that at day 273 postinfection, a significant degree of paracortical hyperplasia was observed only in RMs and was associated with

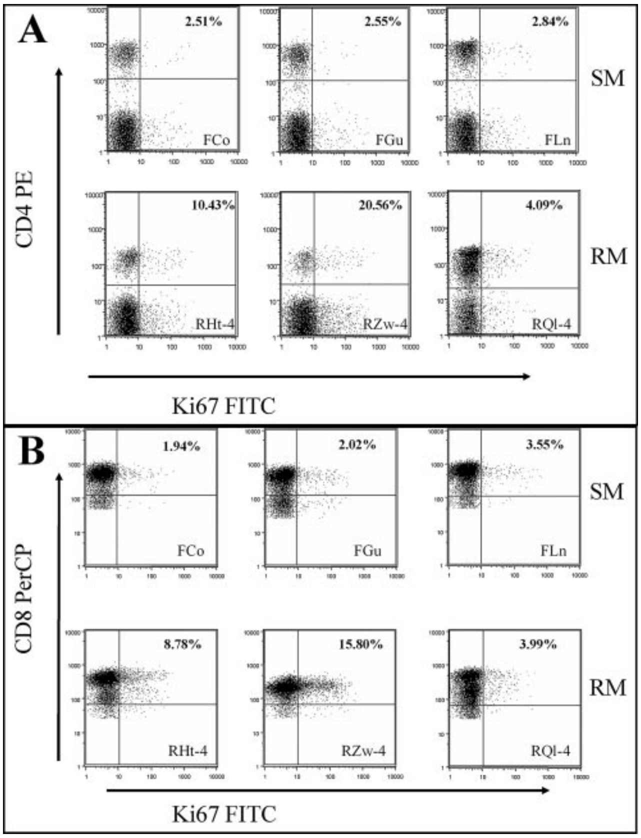


FIG. 5. Persistence of chronic high levels of T-cell proliferation during chronic SIVsm infection of RMs. (A and B) Rates of $CD4^+$ (A) and $CD8^+$ (B) T-cell proliferation as measured by Ki67 expression in three SIVsm-infected SMs and three RMs at day 720 postinfection. The numbers in the dot plots represent the percentages of $Ki67^+$ cells on total $CD4^+$ or $CD8^+$ T lymphocytes.

the presence of numerous lymphoblasts, histiocytosis, and vascular proliferation (Fig. 6A). In contrast, lymph nodes from SIVsm-infected SMs showed a more pronounced follicular hyperplasia with prominent germinal centers (Fig. 6B). To determine whether these different patterns of lymphoid activation were associated with different levels of T-cell apoptosis, we measured the frequency of apoptotic cells by using the TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling) assay. The levels of lymphocyte apoptosis in the paracortex (T-cell-dependent area) in the lymph nodes of SIV-infected RMs were markedly higher than those observed in SMs (Fig. 6C to E). In all, these results indicate that the level of T-cell activation, proliferation, and apoptosis induced by SIVsm-infection are significantly higher in RMs than in SMs.

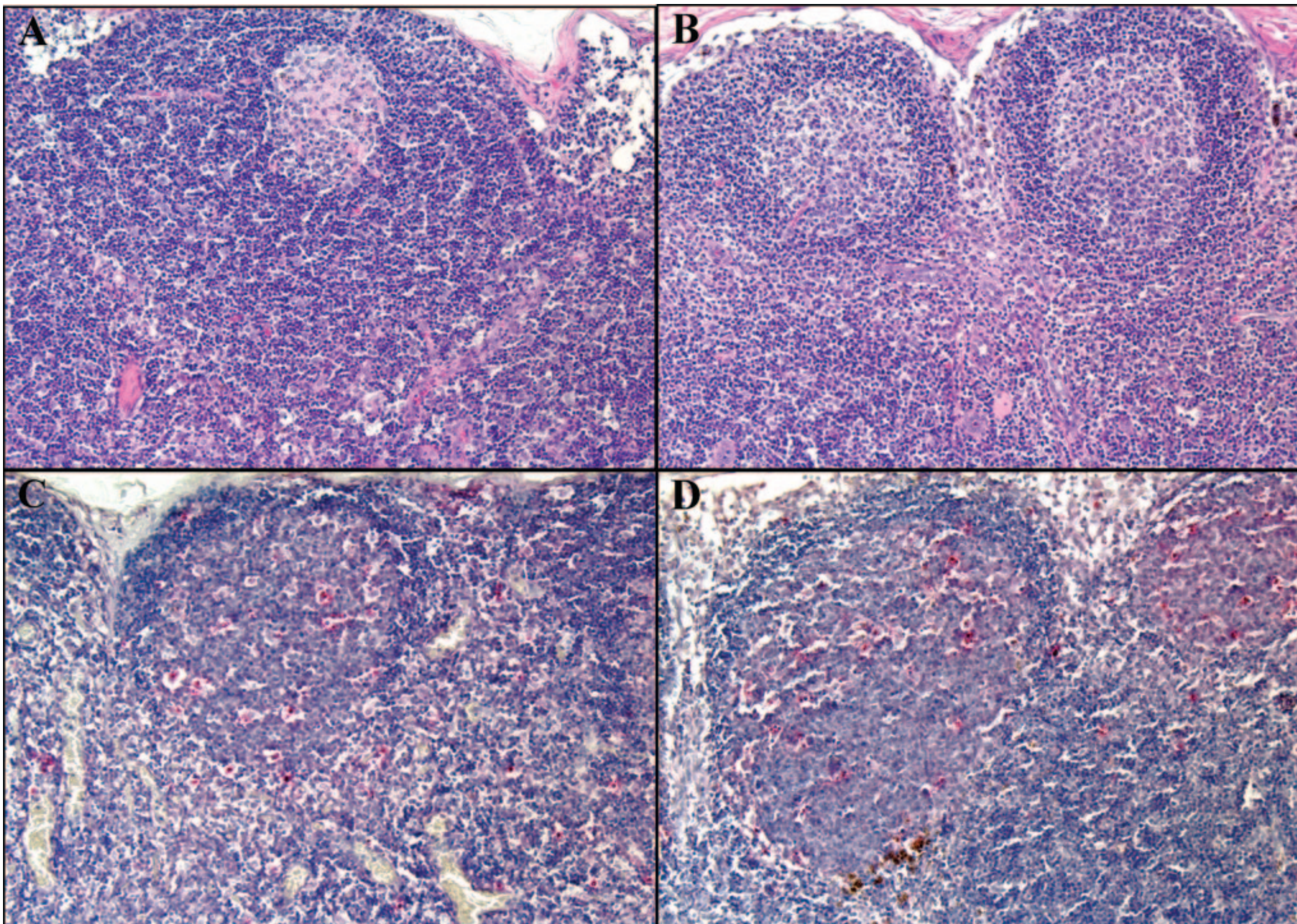
Despite having similar set-point viremia, only RMs experience significant $CD4^+$-T-cell decline and the development of AIDS. Significant decline of $CD4^+$ T cells was observed in two of three RMs (Fig. 7A), whereas no decline was observed in animal RQl4, whose infection was characterized by low viremia and minimal T-cell activation. In contrast, the percentage of $CD4^+$ T cells showed a minimal decline in all SMs (Fig. 7B), with absolute numbers remaining within the normal range. These observations are consistent with the observation that naturally infected SMs display a modest $CD4^+$-T-cell decline despite years of SIVsm infection (5, 53). Animals RHt4 and RZw4 developed AIDS 2.5 and 3.5 years postinfection and were euthanized. Animal RQl4 was euthanized 5 years postinfection without symptoms of AIDS. All three SIV-infected SM were alive and without symptoms at 5 years postinfection. The pathogenic outcomes in RMs infected with uncloned SIVsm were observed without repeated passage of the virus in RMs and recapitulated the immunological perturbations characteristic of pathogenic HIV and SIV infections.

DISCUSSION

This study demonstrated that inoculation of naive SMs and RMs with uncloned SIVsm quasispecies results in early and active viral replication in both species and achieves set-point viremia levels in SMs that are similar to those observed in naturally infected animals. Experimentally infected SMs also mirrored naturally SIV-infected animals in their near-normal $CD4^+$-T-cell counts and absence of immunodeficiency, whereas SIVsm-infected RMs developed AIDS. Thus, infection of SMs and RMs with uncloned SIVsm provides a new and useful model to study the factors that lead to divergent infection outcomes.

Repeated serial passage of SIV in macaques will select for highly adapted viruses with uniformly high replicative capacity in the new host and, along with this, an attendant rapid course of disease (6, 58). However, our observation that SIVsm that has never been passaged in an RM can replicate to high levels and cause disease in this new species demonstrates that neither serial passaging nor an extensive period of SIV adaptation in the new host is required to cause AIDS. The use of a diverse, naturally occurring SIVsm quasispecies inoculum, as opposed to a biologically or molecularly cloned virus, may have enabled the ready infection of the new RM hosts by providing numerous variants, including ones that might be well suited to replicate efficiently in a new host environment. The observed facile adaptation of the naturally occurring SIVsm quasispecies to a new host may help to explain why certain naturally occurring lentiviruses, such as SIVsm, have been so successful at establishing zoonotic infections (L. Demma and S. I. Staprans, unpublished data).

Acute viral infections are usually marked by the activation and expansion of $CD8^+$ T cells. In acute HIV-1, SIV, Epstein-Barr virus, or other viral infections, up to 30 to 60% of peripheral blood $CD8^+$ T cells have been reported to proliferate (12, 27, 51). Phenotypic and functional studies demonstrate that significant numbers of these T cells are virus-specific effector cells (3, 12, 31, 51). The present study of RM and SM responses to SIVsm infection demonstrated a species-specific divergent T-cell response that was evident from the first days of infection. Although rapid activation of T cells was observed in both species, the magnitude of T-cell activation appeared to be higher in the RMs, albeit the small numbers of animals precluded statistical analyses. A clearer dichotomy between the species was evident in terms of T-cell proliferation; RMs showed more extensive acute and persistent proliferation of T cells, especially CD8 T cells, during SIV infection. In contrast, two of three SMs did not manifest any evidence of acute CD8 T-cell proliferation, despite high levels of peak viremia, and a



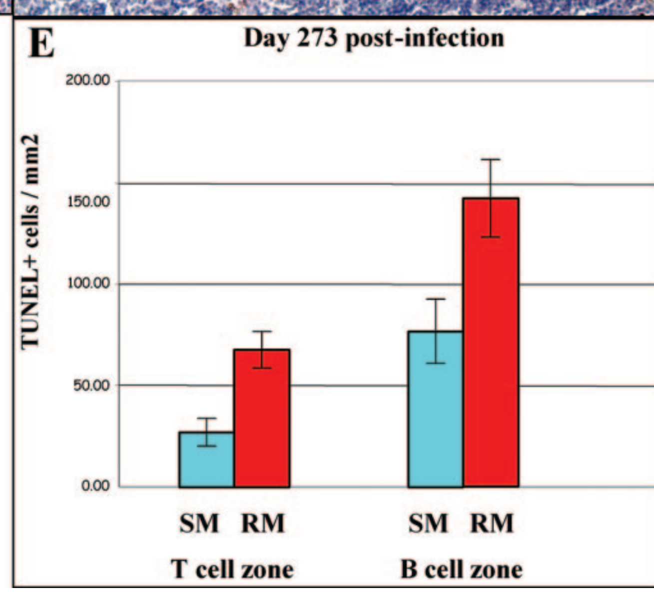


FIG. 6. Elevated T-cell activation and apoptosis in lymph nodes from chronically SIVsm-infected RMs. (A and B) Hematoxylin-eosin staining of a lymph node biopsy obtained from a representative RM (RZw4) (A) and SM (FGu) (B) and at day 273 postinfection. (C and D) Levels of apoptosis, as measured by TUNEL staining, in a lymph node biopsy obtained from a representative RM (RZw4) (C) and SM (FGu) (D) and at day 273 postinfection. (E) Frequency of apoptotic cells in lymph nodes.

third SM manifested a transient increase in proliferating cells. None of the SMs exhibited elevated T-cell proliferation during chronic infection. The absence of significant T-cell proliferation during the acute, highly viremic primary infection period of SMs suggests that SMs do not mount a strong cellular immune response to SIV infection. Conversely, the strong correlation between significant levels of acute $CD8^+$-T-cell proliferation in RMs and the more dramatic postpeak suppression of viremia suggests that virus-specific $CD8^+$-T-cell responses were more active in RMs. The application of SIV-specific cellular immune response assays will be required to corroborate that the observed species-specific differences in acute levels of

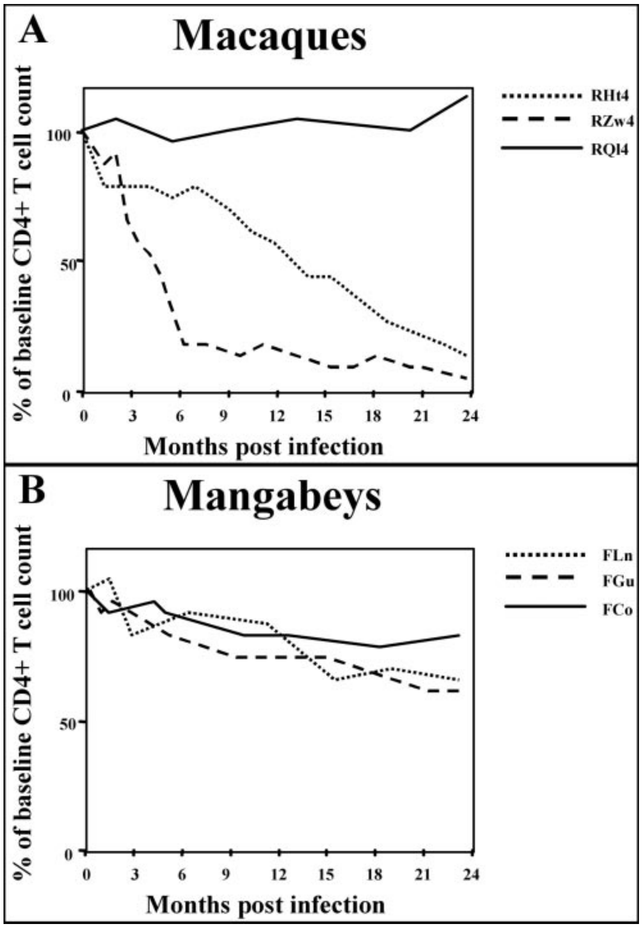


FIG. 7. $CD4^+$ T-cell depletion and AIDS during SIVsm infection of RMs. (A and B) Measurements over time of $CD4^+$ T cells in three SIVsm-infected RMs (A) and three SMs (B) between days 0 and 720 postinfection. Each line represents an individual animal.

proliferating T cells reflect differences in SIV-specific cellular immune responses.

A lack of T-cell proliferation during acute SIV infection of SMs was also evident in the lymph nodes during peak viremia. Instead, a prominent B-cell expansion and the formation of multiple secondary follicles within germinal centers was observed. These observations are consistent with histological analyses of acute SIVagm infection of the natural host species AGMs (9) and suggest a common pattern of early natural host responses to SIV infection that involve little activation of the cellular immune response (41).

Species-specific divergent patterns of T-cell proliferation continued to be evident during chronic infection, with RMs exhibiting higher levels of T-cell proliferation. The absence of elevated T-cell proliferation despite substantial set-point viremia in SMs has been confirmed in a large number of experimentally SIVsm-infected animals (A. Barry and M. B. Feinberg, unpublished results). Thus, experimental SIVsm infection of SMs recapitulates the relatively immune quiescent host-virus equilibrium observed in naturally infected SMs (53).

That SMs do not lose $CD4^+$ T cells despite significant levels of SIV replication demonstrates that SIV replication alone cannot account for $CD4^+$-T-cell loss. Any direct cytopathic effects of SIV infection that SMs experience are not, in and of themselves, sufficient to cause progressive and irreversible damage to the immune system. Rather, it must be the differences in SM and RM responses to SIV infection that lead to divergent disease outcome. Thus, whereas substantial levels of T-cell proliferation were observed in the SIVsm-infected RMs, SMs exhibited modest T-cell activation during the acute infection period and showed little evidence of elevated T-cell proliferation during either acute or chronic infection. If the elevated $Ki67^+$ T cells observed in RMs reflect increased proliferation of effector T cells, as has been demonstrated in HIV-infected humans (22), this would cause the expansion of cells that produce proinflammatory and proapoptotic cytokines that could lead to bystander effects. Indeed, in RMs the partial suppression of SIV replication was accompanied by increased $CD4^+$- and $CD8^+$-T-cell proliferation and increased lymphocyte apoptosis. These data suggest that circumstances of persistent SIV antigenemia in nonnatural hosts result in chronic immune system activation, evidenced by increased T-cell proliferation and apoptosis, that contribute to CD4 T-cell loss. To elucidate the role of SIV-specific cellular immune responses in this immunopathology will require the successful inhibition or elicitation of such responses in nonnatural and natural host species, respectively, prior to or during primary SIV infection (12). Studies of SIV-specific humoral responses in natural and nonnatural host species will be required to determine the role of antibodies, if any, in the observed divergent disease outcomes.

The present study indicates that establishment of the nonpathogenic SM-SIVsm equilibrium differs from the manner in which certain lymphocytic choriomeningitis virus strains establish CD8 T-cell anergy in mice through an initial CD8 T-cell expansion, followed by the subsequent exhaustion of virus-specific CD8 CTLs (41). The apparent absence of a substantive T-cell response from the first days of SIV infection of SMs suggests a break between the innate and adaptive immune responses or an altered innate response to infection. This new information informs ongoing follow-up studies, which suggest altered natural killer and dendritic cell responses to SIV infection of SMs (A. Barry, S. I. Staprans, and M. B. Feinberg, unpublished results). Ongoing studies are aimed at dissecting the cellular and molecular basis of the observed divergent, species-specific responses to SIVsm and whether SMs also mount subdued responses to other cellular pathogens. SMs are not obviously compromised in their ability to mount cellular immune responses to other pathogens and do not experience elevated rates of disease after infection with persistent pathogens, such as cytomegalovirus and STLV-1 (28; H. McClure, unpublished data). An intriguing exception is that, in contrast to RMs and humans, SMs readily succumb to lepromatous leprosy (17, 18), perhaps reflecting inadequate induction of an innate inflammatory or Th1-biased adaptive immune response (32, 39).

In summary, this comparative study of RMs and SMs infected with SIVsm suggests that SMs are able to maintain normal $CD4^+$-T-cell levels despite substantial virus replication by virtue of their attenuated cellular immune responses to SIV. An absence of significant T-cell proliferation is evident from the first days of infection, apparently avoiding the establishment of a state of chronic generalized immune activation that would elicit deleterious bystander effects, such as those observed in HIV infection of humans. The species-specific responses observed in the present study demonstrate how host factors are critical in determining the divergent outcome of SIVsm infection in natural and nonnatural hosts. The increasing recognition that HIV-induced AIDS in humans may result from host responses that can eventually cause immunopathology provides new avenues for investigation and may lead to the identification of immunomodulatory interventions that inhibit harmful host responses to HIV infection.

ACKNOWLEDGMENTS

We thank F. Novembre and S. Garg for technical assistance and D. Anderson for assistance in the pathology studies.

This study was supported by NIH grants AI49155 and AI44763 to M.B.F., grant RR00165 to the Yerkes National Primate Research Center, and grant AI50409 to the Emory CFAR.

REFERENCES

1. **Borrow, P., H. Lewicki, B. H. Hahn, G. M. Shaw, and M. B. Oldstone.** 1994. Virus-specific $CD8^+$ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J. Virol. **68:**6103–6110.
2. **Broussard, S. R., S. I. Staprans, R. White, E. M. Whitehead, M. B. Feinberg, and J. S. Allan.** 2001. Simian immunodeficiency virus replicates to high levels in naturally infected African green monkeys without inducing immunologic or neurologic disease. J. Virol. **75:**2262–2275.
3. **Callan, M. F., L. Tan, N. Annels, G. S. Ogg, J. D. Wilson, C. A. O'Callaghan, N. Steven, A. J. McMichael, and A. B. Rickinson.** 1998. Direct visualization of antigen-specific $CD8^+$ T cells during the primary immune response to Epstein-Barr virus in vivo. J. Exp. Med. **187:**1395–1402.
4. **Carlson, J. R., T. P. McGraw, E. Keddie, J. L. Yee, A. Rosenthal, A. J. Langlois, R. Dickover, R. Donovan, P. A. Luciw, and M. B. Jennings.** 1990. Vaccine protection of rhesus macaques against simian immunodeficiency virus infection. AIDS Res. Hum. Retrovir. **6:**1239–1246.
5. **Chakrabarti, L. A., S. R. Lewin, L. Zhang, A. Gettie, A. Luckay, L. N. Martin, E. Skulsky, D. D. Ho, C. Cheng-Mayer, and P. A. Marx.** 2000. Normal T-cell turnover in sooty mangabeys harboring active simian immunodeficiency virus infection. J. Virol. **74:**1209–1223.
6. **Chen, Z., Y. Huang, X. Zhao, E. Skulsky, D. Lin, J. Ip, A. Gettie, and D. D. Ho.** 2000. Enhanced infectivity of an R5-tropic simian/human immunodeficiency virus carrying human immunodeficiency virus type 1 subtype C envelope after serial passages in pig-tailed macaques (*Macaca nemestrina*). J. Virol. **74:**6501–6510.
7. **Chen, Z., P. Telfier, A. Gettie, P. Reed, L. Zhang, D. D. Ho, and P. A. Marx.** 1996. Genetic characterization of new West African simian immunodeficiency virus SIVsm: geographic clustering of household-derived SIV strains with human immunodeficiency virus type 2 subtypes and genetically diverse viruses from a single feral sooty mangabey troop. J. Virol. **70:**3617–3627.
8. **Deeks, S. G., and B. D. Walker.** 2004. The immune response to AIDS virus infection: good, bad, or both? J. Clin. Investig. **113:**808–810.
9. **Diop, O. M., A. Gueye, M. Dias-Tavares, C. Kornfeld, A. Faye, P. Ave, M. Huerre, S. Corbet, F. Barre-Sinoussi, and M.C. Muller-Trutwin.** 2000. High levels of viral replication during primary simian immunodeficiency virus SIVagm infection are rapidly and strongly controlled in African green monkeys. J. Virol. **74:**7538–7547.
10. **Finkel, T. H., G. Tudor-Williams, N. K. Banda, M. F. Cotton, T. Curiel, C. Monks, T. W. Baba, R. M. Ruprecht, and A. Kupfer.** 1995. Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nat. Med. **1:**129–134.
11. **Gao, F., E. Bailes, D. L. Robertson, Y. Chen, C. M. Rodenburg, S. F. Michael, L. B. Cummins, L. O. Arthur, M. Peeters, G. M. Shaw, P. M. Sharp, and B. H. Hahn.** 1999. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. Nature **397:**436–441.
12. **Garber, D. A., G. Silvestri, A. P. Barry, A. Fedanov, N. Kozyr, H. McClure, D. C. Montefiori, C. P. Larsen, J. D. Altman, S. I. Staprans, and M. B. Feinberg.** 2004. Blockade of T-cell costimulation reveals interrelated actions of $CD4^+$ and $CD8^+$ T cells in control of SIV replication. J. Clin. Investig. **113:**836–845.
13. **Gea-Banacloche, J. C., S. A. Migueles, L. Martino, W. L. Shupert, A. C. McNeil, M. S. Sabbaghian, L. Ehler, C. Prussin, R. Stevens, L. Lambert, J. Altman, C. W. Hallahan, J. C. deq Uiros, and M. Connors.** 2000. Maintenance of large numbers of virus-specific $CD8^+$ T cells in HIV-infected progressors and long-term nonprogressors. J. Immunol. **165:**1082–1092.
14. **Gerdes, J., H. Lemke, H. Baisch, H. H. Wacker, U. Schwab, and H. Stein.** 1984. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J. Immunol. **133:**1710–1715.
15. **Giorgi, J. V., L. E. Hultin, J. A. McKeating, T. D. Johnson, B. Owens, L. P. Jacobson, R. Shih, J. Lewis, D. J. Wiley, J. P. Phair, S. M. Wolinsky, and R. Detels.** 1999. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. J. Infect. Dis. **179:**859–870.
16. **Giorgi, J. V., R. H. Lyles, J. L. Matud, T. E. Yamashita, J. W. Mellors, L. E. Hultin, B. D. Jamieson, J. B. Margolick, C. R. Rinaldo, Jr., J. P. Phair, R. Detels, and A. C. S. Multicenter.** 2002. Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection. J. Acquir. Immune Defic. Syndr. **29:**346–355.
17. **Gormus, B. J., K. Xu, G. B. Baskin, L. N. Martin, R. P. Bohm, J. L. Blanchard, P. A. Mack, M. S. Ratterree, H. M. McClure, and W. M. Meyers.** 1995. Experimental leprosy in monkeys. I. Sooty mangabey monkeys: transmission, susceptibility, clinical and pathological findings. Leprosy Rev. **66:**96–104.
18. **Gormus, B. J., K. Xu, S. N. Cho, G. B. Baskin, R. P. Bohm, L. N. Martin, J. L. Blanchard, P. A. Mack, M. S. Ratterree, and W. M. Meyers.** 1995. Experimental leprosy in monkeys. II. Longitudinal serological observations in sooty mangabey monkeys. Leprosy Rev. **66:**105–125.
19. **Gougeon, M. L., H. Lecoeur, A. Dulioust, M. G. Enouf, M. Crouvoiser, C. Goujard, T. Debord, and L. Montagnier.** 1996. Programmed cell death in peripheral lymphocytes from HIV-infected persons: increased susceptibility to apoptosis of CD4 and CD8 T cells correlates with lymphocyte activation and with disease progression. J. Immunol. **156:**3509–3520.
20. **Grossman, Z., M. Meier-Schellersheim, A. E. Sousa, R. M. Victorino, and W. E. Paul.** 2002. $CD4^+$ T-cell depletion in HIV infection: are we closer to understanding the cause? Nat. Med. **8:**319–323.
21. **Hazenberg, M. D., S. A. Otto, B. H. van Benthem, M. T. Roos, R. A. Coutinho, J. M. Lange, D. Hamann, M. Prins, and F. Miedema.** 2003. Persistent immune activation in HIV-1 infection is associated with progression to AIDS. AIDS **17:**1881–1888.
22. **Hellerstein, M. K., R. A. Hoh, M. B. Hanley, D. Cesar, D. Lee, R. A. Neese, and J. M. McCune.** 2003. Subpopulations of long-lived and short-lived T cells in advanced HIV-1 infection. J. Clin. Investig. **112:**956–966.
23. **Hirsch, V. M., G. Dapolito, P. R. Johnson, W. R. Elkins, W. T. London, R. J. Montali, S. Goldstein, and C. Brown.** 1995. Induction of AIDS by simian immunodeficiency virus from an African green monkey: species-specific variation in pathogenicity correlates with the extent of in vivo replication. J. Virol. **69:**955–967.
24. **Ho, D. D., A. U. Neumann, A. S. Perelson, W. Chen, J. M. Leonard, and M. Markowitz.** 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature **373:**123–126.
25. **Hunt, P. W., J. N. Martin, E. Sinclair, B. Bredt, E. Hagos, H. Lampiris, and S. G. Deeks.** 2003. T cell activation is associated with lower $CD4^+$ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. J. Infect. Dis. **187:**1534–1543.
26. **Kaur, A., R. M. Grant, R. E. Means, H. McClure, M. Feinberg, and R. P. Johnson.** 1998. Diverse host responses and outcomes following simian immunodeficiency virus SIVmac239 infection in sooty mangabeys and rhesus macaques. J. Virol. **72:**9597–9611.
27. **Kaur, A., C. L. Hale, S. Ramanujan, R. K. Jain, and R. P. Johnson.** 2000. Differential dynamics of $CD4^+$ and $CD8^+$ T-lymphocyte proliferation and activation in acute simian immunodeficiency virus infection. J. Virol. **74:**8413–8424.
28. **Kaur, A., N. Kassis, C. L. Hale, M. Simon, M. Elliott, A. Gomez-Yafal, J. D. Lifson, R. C. Desrosiers, F. Wang, P. Barry, M. Mach, and R. P. Johnson.** 2003. Direct relationship between suppression of virus-specific immunity and emergence of cytomegalovirus disease in simian AIDS. J. Virol. **77:**5749–5758.
29. **Kaur, A., J. Yang, D. Hempel, L. Gritz, G. P. Mazzara, H. McClure, and R. P. Johnson.** 2000. Identification of multiple simian immunodeficiency virus (SIV)-specific CTL epitopes in sooty mangabeys with natural and experimentally acquired SIV infection. J. Immunol. **164:**934–943.
30. **Koup, R. A., J. T. Safrit, Y. Cao, C. A. Andrews, G. McLeod, W. Borkowsky, C. Farthing, and D. D. Ho.** 1994. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J. Virol. **68:**4650–4655.
31. **Kuroda, M. J., J. E. Schmitz, W. A. Charini, C. E. Nickerson, M. A. Lifton, C. I. Lord, M. A. Forman, and N. L. Letvin.** 1999. Emergence of CTL coincides with clearance of virus during primary simian immunodeficiency virus infection in rhesus monkeys. J. Immunol. **162:**5127–5133.
32. **Levin, M., and M. Newport.** 1999. Understanding the genetic basis of susceptibility to mycobacterial infection. Proc. Assoc. Am. Phys. **111:**308–312.
33. **Little, S. J., A. R. McLean, C. A. Spina, D. D. Richman, and D. V. Havlir.** 1999. Viral dynamics of acute HIV-1 infection. J. Exp. Med. **190:**841–850.
34. **McCune, J. M.** 2001. The dynamics of $CD4^+$ T-cell depletion in HIV disease. Nature **410:**974–979.
35. **Mellors, J. W., A. Munoz, J. V. Giorgi, J. B. Margolick, C. J. Tassoni, P. Gupta, L. A. Kingsley, J. A. Todd, A. J. Saah, R. Detels, J. P. Phair, and C. R.**

Rinaldo, Jr. 1997. Plasma viral load and CD4$^+$ lymphocytes as prognostic markers of HIV-1 infection. Ann. Intern. Med. **126:**946–954.

36. **Mellors, J. W., C. R. Rinaldo, Jr., P. Gupta, R. M. White, J. A. Todd, and L. A. Kingsley.** 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science **272:**1167–1170. (Erratum, **275:**14, 1997.)
37. **Meyaard, L., S. A. Otto, R. R. Jonker, M. J. Mijnster, R. P. Keet, and F. Miedema.** 1992. Programmed death of T cells in HIV-1 infection. Science **257:**217–219.
38. **Migueles, S. A., A. C. Laborico, W. L. Shupert, M. S. Sabbaghian, R. Rabin, C. W. Hallahan, D. Van Baarle, S. Kostense, F. Miedema, M. McLaughlin, L. Ehler, J. Metcalf, S. Liu, and M. Connors.** 2002. HIV-specific CD8$^+$ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. Nat. Immunol. **3:**1061–1068.
39. **Modlin, R. L.** 1994. Th1-Th2 paradigm: insights from leprosy. J. Investig. Dermatol. **102:**828–832.
40. **Mohri, H., A. S. Perelson, K. Tung, R. M. Ribeiro, B. Ramratnam, M. Markowitz, R. Kost, A. Hurley, L. Weinberger, D. Cesar, M. K. Hellerstein, and D. D. Ho.** 2001. Increased turnover of T lymphocytes in HIV-1 infection and its reduction by antiretroviral therapy. J. Exp. Med. **194:**1277–1287.
41. **Moskophidis, D., F. Lechner, H. Pircher, and R. M. Zinkernagel.** 1993. Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. Nature **362:**758–761. (Erratum, **364:**262.)
42. **Muro-Cacho, C. A., G. Pantaleo, and A. S. Fauci.** 1995. Analysis of apoptosis in lymph nodes of HIV-infected persons. Intensity of apoptosis correlates with the general state of activation of the lymphoid tissue and not with stage of disease or viral burden. J. Immunol. **154:**5555–5566.
43. **National Institutes of Health.** 1985. Guide for the care and use of laboratory animals, rev. ed. Department of Health and Human Services publication 85-23. National Institutes of Health, Bethesda, Md.
44. **Nowak, M. A., A. L. Lloyd, G. M. Vasquez, T. A. Wiltrout, L. M. Wahl, N. Bischofberger, J. Williams, A. Kinter, A. S. Fauci, V. M. Hirsch, and J. D. Lifson.** 1997. Viral dynamics of primary viremia and antiretroviral therapy in simian immunodeficiency virus infection. J. Virol. **71:**7518–7525.
45. **Ou, R., S. Zhou, L. Huang, and D. Moskophidis.** 2001. Critical role for alpha/beta and gamma interferons in persistence of lymphocytic choriomeningitis virus by clonal exhaustion of cytotoxic T cells. J. Virol. **75:**8407–8423.
46. **Peeters, M., V. Courgnaud, B. Abela, P. Auzel, X. Pourrut, F. Bibollet-Ruche, S. Loul, F. Liegeois, C. Butel, D. Koulagna, E. Mpoudi-Ngole, G. M. Shaw, B. Hahn, and E. Delaporte.** 2002. Risk to human health from a plethora of simian immunodeficiency viruses in primate bushmeat. Emerg. Infect. Dis. **8:**451–457.
47. **Pitcher, C. J., S. I. Hagen, J. M. Walker, R. Lum, B. L. Mitchell, V. C. Maino, M. K. Axthelm, and L. J. Picker.** 2002. Development and homeostasis of T-cell memory in rhesus macaque. J. Immunol. **168:**29–43.
48. **Regoes, R. R., R. Antia, D. A. Garber, G. Silvestri, M. B. Feinberg, and S. I. Staprans.** 2004. Roles of target cells and virus-specific cellular immunity in primary simian immunodeficiency virus infection. J. Virol. **78:**4866–4875.
49. **Rey-Cuille, M. A., J. L. Berthier, M. C. Bomsel-Demontoy, Y. Chaduc, L. Montagnier, A. G. Hovanessian, and L. A. Chakrabarti.** 1998. Simian immunodeficiency virus replicates to high levels in sooty mangabeys without inducing disease. J. Virol. **72:**3872–3886.
50. **Roederer, M.** 1998. Getting to the HAART of T cell dynamics. Nat. Med. **4:**145–146.
51. **Roos, M. T., R. A. van Lier, D. Hamann, G. J. Knol, I. Verhoofstad, D. van Baarle, F. Miedema, and P. T. Schellekens.** 2000. Changes in the composition of circulating CD8$^+$ T cell subsets during acute Epstein-Barr and human immunodeficiency virus infections in humans. J. Infect. Dis. **182:**451–458.
52. **Silvestri, G., and M. B. Feinberg.** 2003. Turnover of lymphocytes and conceptual paradigms in HIV infection. J. Clin. Investig. **112:**821–824.
53. **Silvestri, G., D. L. Sodora, R. A. Koup, M. Paiardini, S. P. O'Neil, H. M. McClure, S. I. Staprans, and M. B. Feinberg.** 2003. Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia. Immunity **18:**441–452.
54. **Sousa, A. E., J. Carneiro, M. Meier-Schellersheim, Z. Grossman, and R. M. Victorino.** 2002. CD4 T-cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. J. Immunol. **169:**3400–3406.
55. **Staprans, S., B. Corliss, J. Guthrie, and M. B. Feinberg.** 1996. Quantitative methods to monitor viral load in simian immunodeficiency virus infections, p. 167–184. *In* K. Adolph (ed.), Viral genome methods. CRC Press, Boca Raton, Fla.
56. **Staprans, S. I., A. P. Barry, G. Silvestri, J. T. Safrit, N. Kozyr, B. Sumpter, H. Nguyen, H. McClure, D. Montefiori, J. I. Cohen, and M. B. Feinberg.** 2004. Enhanced SIV replication and accelerated progression to AIDS in macaques primed to mount a CD4 T cell response to the SIV envelope protein. Proc. Natl. Acad. Sci. USA **101:**13026–13031.
57. **Staprans, S. I., P. Dailey, J., A. Rosenthal, C. Horton, R. M. Grant, N. Lerche, and M. B. Feinberg.** 1999. Simian immunodeficiency virus disease course is predicted by the extent of virus replication during primary infection. J. Virol. **73:**4829–4839.
58. **Valli, P. J., and J. Goudsmit.** 1998. Structured-tree topology and adaptive evolution of the simian immunodeficiency virus SIVsm envelope during serial passage in rhesus macaques according to likelihood mapping and quartet puzzling. J. Virol. **72:**3673–3683.
59. **Vogel, T. U., M. R. Reynolds, D. H. Fuller, K. Vielhuber, T. Shipley, J. T. Fuller, K. J. Kunstman, G. Sutter, M. L. Marthas, V. Erfle, S. M. Wolinsky, C. Wang, D. B. Allison, E. W. Rud, N. Wilson, D. Montefiori, J. D. Altman, and D. I. Watkins.** 2003. Multispecific vaccine-induced mucosal cytotoxic T lymphocytes reduce acute-phase viral replication but fail in long-term control of simian immunodeficiency virus SIVmac239. J. Virol. **77:**13348–13360.
60. **Watson, A., J. Ranchalis, B. Travis, J. McClure, W. Sutton, P. R. Johnson, S. L. Hu, and N. L. Haigwood.** 1997. Plasma viremia in macaques infected with simian immunodeficiency virus: plasma viral load early in infection predicts survival. J. Virol. **71:**284–290.
61. **Wei, X., S. K. Ghosh, M. E. Taylor, V. A. Johnson, E. A. Emini, P. Deutsch, J. D. Lifson, S. Bonhoeffer, M. A. Nowak, and B. H. Hahn.** 1995. Viral dynamics in human immunodeficiency virus type 1 infection. Nature **373:**117–122.
62. **Zhang, Z., T. Schuler, M. Zupancic, S. Wietgrefe, K. Staskus, K. Reimann, T. Reinhart, M. Rogan, W. Cavert, C. Miller, R. Veazey, D. Notermans, S. Little, S. Danner, D. Richman, D. Havlir, J. Wong, H. Jordan, T. Schacker, P. Racz, K. Tenner-Racz, N. Letvin, S. Wolinsky, and A. Haase.** 1999. Sexual transmission and propagation of SIV and HIV in resting and activated CD4$^+$ T cells. Science **286:**1353–1357.